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STUDIES ON BARLEY SMUTS AND ON LOOSE SMUT OF WHEAT

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ABSTRACT

In field and greenhouse collections of barley smut five types intermediate between *Ustilago hordei* and *U. nuda* were identified, of which Type 4 corresponds closely to *U. medians* Biedenkopf and *U. nigra* Tapke. Types 2 and 3 are intermediate between Type 4 and *U. hordei* and Types 5 and 6 intermediate between Type 4 and *U. nuda.*

Alpha barley was infected by all smut collections used in these experiments; Featherston barley was not infected by certain Type 4 collections, Tennessee Winter barley by *U. nuda* (Featherston collection), Reward wheat by *U. tritici* (Honor form) nor Honor wheat by *U. tritici* (Reward form).

A micromanipulator for making monosporidial cultures was devised.

Type 3 and *U. hordei* were hybridized by inoculating dehulled seed with a monosporidial culture from each.

Seed from flowers inoculated with *U. nuda* was inoculated with different bisporidial cultures of *U. hordei* or Type 3. From these inoculations, plants were obtained as follows: Heads infected with *U. nuda, U. hordei,* or Type 3 only; part of the heads infected with *U. nuda* and part with *U. hordei* or Type 3; heads infected with a mixture of *U. nuda* and Type 3 or of *U. nuda* and *U. hordei* in the same head or, in the case of two plants from one culture, part of the heads contained smooth spores germinating by promycelia and sporidia (Type 3), echinulate spores germinating by germ tubes (*U. nuda*), and echinulate spores germinating by promycelia and sporidia. These latter are suspected hybrid spores between Type 3 and *U. nuda.*

Mycelium of Type 4 was not observed to have penetrated "flower-inoculated" seed. The resting sporidia and hyphae which developed on the surface of the caryopsis were observed to produce sporidia within 24 hours after the seed was put to germinate.

Abundant mycelium was present in seed of Honor wheat "flower-inoculated" with the Honor form of *U. tritici,* of Reward wheat inoculated with the Reward form, and of Alpha and Featherston barley inoculated with *U. nuda.*

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STUDIES ON BARLEY SMUTS AND ON LOOSE SMUT OF WHEAT[1]

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INTRODUCTION

As indicated by brief surveys made in 1932 and 1933, barley smut is very prevalent in barley fields in the neighborhood of Geneva, N. Y. Kirby (1927)[2] states that loose smut is the most destructive barley disease in New York State, causing an annual loss of 2 per cent and not uncommonly as much as 10 per cent. He found Alpha, a two-rowed barley commonly grown in this neighborhood, to be particularly susceptible.

A detailed study of the causal organisms seemed advisable in developing adequate control measures, since barley varieties have been observed to respond differently to inoculation by loose smut and since floral and seedling infection have both been shown to occur and since contradictory evidence has been accumulating in regard to the value of dusting with various chemicals as a control for loose smut.

HISTORICAL REVIEW

The researches of Brefeld (1888), Jensen (1888), and Kellerman and Swingle (1890) showed that there are two smuts of barley, *Ustilago nuda* (Jens.) Kell. and Sw., loose smut, and *Ustilago hordei* (Pers.) Kell. and Sw., covered smut.

In 1903, Brefeld infected both wheat and barley by inoculating flowers with loose smut of wheat and barley, respectively. This was confirmed for barley loose smut by the further inoculation studies

[1] This paper represents only an incompleted series of observations which seem advisable to publish at this time, since work on this project is being discontinued by the writer. The writer wishes to thank Prof. M. F. Barrus of Cornell University for suggesting the problem and for counsel and criticism thruout the course of the work, all those who kindly furnished collections of spores and seeds, and the Division of Pomology of the New York State Agricultural Experiment Station for equipment, supplies, and working space.

[2] Refers to Literature Cited, page 35.

of Brefeld and Falck (1905), Hecke (1904), and others, and by the cytological work of Hecke (1905), Brioli (1910), Brioli and Schikkorra (1913), and Lang (1917), who found the mycelium in the seeds of flowers which had been inoculated.

Seedling infection was shown for covered smut of barley by Jensen (1888) and for oat smut by Brefeld (1895) who at the same time carefully followed the penetration of the mycelium into the seedling tissue. Tisdale (1923) and others have found that seedling infection is increased by dehulling the seed. Faris (1924B) produced abundant infection with *U. hordei* without dehulling the seed, but under conditions of controlled moisture and soil acidity and at a temperature of 15° to 20° C.

Physiologic forms of the barley smuts similar to those in other fungi have been found. Thus, Faris (1924A), on the basis of host reaction, identified five physiologic forms of *U. hordei* while Rodenhiser (1926, 1928), on the basis of cultural characteristics, identified 12 physiologic forms of *U. nuda* and 7 of *U. hordei.* Rodenhiser (1928) observed greater differences in different cultures of *U. nuda* than between *U. nuda* and *U. tritici* (Pers.) Jens.

During the same time results have been steadily accumulating which show that either there are species of barley smuts other than those described, or that loose smut consists of forms which infect during the seedling stage as well as forms which infect at flowering time.

Biedenkopf, in 1894, collected near Halle, a. S. Germany, smutted barley which in head appearance resembled *U. nuda.* The spores from these heads were echinulate, as are those of *U. nuda,* but germinated by a four-celled promycelium which cut off sporidia, as do those of *U. hordei.* When Biedenkopf found that the chlamydospores germinated by promycelia and sporidia as well as by germ tubes, he thought he must have a mixture of *U. hordei* and *U. nuda,* but on examination he found that "samtliche Sporen Skulptur zeigten." The germinations were performed in extreme July heat under which conditions even spores of *U. hordei* may germinate by germ tubes (Hüttig, 1931; and others). In consequence Biedenkopf considered this smut to be a new species which he named *Ustilago medians* to indicate its morphologic position.

Ustilago medians Biedenkopf was mentioned by Herzberg (1895) and others but does not seem to have been recognized again as such.

Tapke (1932), however, described a new loose smut of barley, that infects seedlings, which he named *Ustilago nigra.* Vanderwalle (1932) found a loose smut which he called "charbon nu tardif." Ruttle (1933) described a series of smuts intermediate between *U. nuda* and *U. hordei,* the outcome of studies begun at this station in 1931, (Hedrick, 1932).

In this period between 1894 and 1932, contradictory results were obtained by inoculating seed with loose smut. In 1914, Johnson reported control of loose smut of barley by treating the seed with formalin. Tisdale, Taylor, and Griffiths (1923), Tisdale and Leukel (1924), and others have found that loose smut can be much reduced with such seed disinfectants as the organic mercury compounds (Chlorophol, Semesan, Germisan, Upsulun, Corona 620), copper carbonate, and formaldehyde, as well as the classical hot water treatment.

To check these results further, Tisdale and Tapke (1924) performed a series of inoculations on six varieties of barley, with loose smut collected on Tennessee Winter barley. Using dehulled seed for seedling-inoculations, they obtained heavy infection on all but Nakano Wase. Floral inoculations gave slight infection on all but Han River and Nakano Wase.

In 1927, Tisdale and Griffiths showed that variants exist in *U. nuda,* some of which seem to infect only thru the flower while others infect thru the seed.

Leukel (1932) states that dust fungicides control loose smut only in those varieties in which seedling infection evidently takes place, such as Tennessee Winter No. 52 and Wisconsin Pedigree No. 5. In varieties in which floral infection apparently takes place, the hot water treatment is the only known control.

A bisexual condition was first shown to exist in the smut fungi by Kniep in 1919, who found that in *U. violacea* fusions occurred between sporidia of one sex group and those of an opposite group but not between sporidia within a given sex group regardless of whether the group was derived from one or more sporidia. Since 1919 this condition has been shown to exist in the majority of the smuts examined. A multisexual condition has been found in *U. longissima* (Bauch 1931 and earlier), in *Sphacelotheca schweinfurthiana* (Bauch 1932) and in *Ustilago zeae* (Stakman et al) (1929) which Bauch (1931, 1932) considers may rest on a combination of sexual and sterility factors.

Owing to the possibility of producing fusions between sporidia of different sex or sterility groups, crosses between different physiologic forms (Goldschmidt, 1928, in *U. violacea*; Stakman, et al, 1929, in *U. zeae*) have been made, as well as crosses between different species. Some of the species crosses were carried only to the point of observing fusions between sporidia or monosporidial mycelium of opposite sex from different species (Dickinson, 1927, *U. hordei* x *U. levis;* Kniep, 1926, *U. bromivora* x *U. hordei, U. tragopogonis* x *U. cardui*; and others) and between sporidia of one species and cells of the germ tube of another species (Kniep, 1926, *U. bromivora* x *U. nuda, U. hordei* x *tritici*; and other fungi). Other crosses have been made, the host inoculated, and the F_1 and following generation studied (Holton, 1932, *U. avenae* x *U. levis*; and others).

TYPES OF BARLEY SMUT

An examination of barley smut collected near Geneva, N. Y., in 1931, 1932, and 1933, and in the greenhouse in 1933, and received from other parts of the United States and Canada revealed the presence not only of typical loose smut (*U. nuda*) and of typical covered smut (*U. hordei*), but also of five intermediate types (Table 1 and Fig. 1), each of which possesses some character common to both smuts. The following criteria were used to separate the types: Appearance of smutted heads, covered or loose; spore masses in sori, compact or loose, darker or lighter shades of brown; spore wall, echinulate or smooth; spore germination, germ tube or promycelium and sporidia. Type of infection was used to a lesser extent. Size of spores was not used as a criterion, since this varied so considerably from head to head within the same type, the variation being as great or greater than the difference in size between types.

Spores were germinated at 20° to 22° C on potato dextrose agar on sterile cover slips placed over Van Tieghem cells in moist sterile petri dishes (Hanna, 1928). They were examined 16 to 24 hours after being put to germinate and, if germination was poor, were examined again 24 to 48 hours later. All material was first examined over a Van Tieghem cell without staining. Certain cover slips were then dried for 10 to 15 minutes and mounted in Linder's lacto-phenol made bright pink by the addition of a few drops of Ehrlich's tri-acid fuchsin. Germination, so far as studied, appears constant under similar conditions of temperature, light, and moisture.

TABLE 1.—SUMMARY OF TYPES OF BARLEY SMUT OBTAINED FROM AN EXAMINATION OF AFFECTED HEADS COLLECTED IN FIELDS NEAR GENEVA, N. Y., IN 1932 AND 1933 AND THOSE RECEIVED FROM VARIOUS SOURCES.

No.*	Appearance of heads	Spore mass		Individual spores		Species or type
		Compactness	Color	Wall	Germination	
1	Covered	Compact	Very dark chocolate brown	Smooth	Promycelia which cut off sporidia	*U. hordei* (Type 1)
2	Covered	Less compact	Very dark chocolate brown	Echinulate	Promycelia which cut off sporidia	Type 2
3	Loose	Powdery to compact	Very dark chocolate brown	Smooth	Promycelia which cut off sporidia	*Charbon nu iardif* ? Type 3 *U. nigra* ?
4	Loose	Powdery to compact	Dark to mid-chocolate brown	Faintly to distinctly echinulate	Promycelia which cut off sporidia	Type 4 *U. medians* ? *U. nigra* ?
5	Loose	Powdery	Olivaceous-brown to mid-brown	Echinulate	Promycelia which cut off sporidia	Type 5 *U. medians* ?
6	Loose	Powdery	Mid-chocolate brown	Echinulate	Germ tubes	Type 6 *U. nuda* ?
7	Loose	Powdery	Olivaceous brown	Echinulate	Germ tubes	*U. nuda* (Type 7)

* Nos. 3 to 7 described as "loose", in early stages of development are covered by a thin membrane which is soon dissipated.

Descriptions of *U. hordei* (Type 1) and *U. nuda* (Type 7) are regarded as unnecessary except as included in other descriptions for comparison.

Type 2.—The covered sori (Fig. 1, 2), spore color in mass, spore germination by promycelia which cut off sporidia (Fig. 2, A), and seedling infection resemble *U. hordei*, but the spore masses are less compact than is usual in *U. hordei* and the spore wall echinulate as in *U. nuda*.

Type 3.—The spore color in mass, spore germination (Fig. 2, B), spore wall, and seedling infection are all similar to *U. hordei*, but the sori membranes are temporary (Fig. 1, 3) as in *U. nuda* or only slightly more permanent, never definitely permanent as in *U. hordei* and Type 2.

Type 4.—Spore germination (Fig. 2, C) is by a promycelium and sporidia as in *U. hordei* but multiplication of sporidia is often less vigorous and rapid. Spore color in mass is usually dark brown but varies from head to head ranging from a color almost, if not quite, as dark as that of *U. hordei* and Types 2 and 3 to a brown so pale that

it is indistinguishable from that in darker-spored heads of *U. nuda.* The membrane is typically thin and evanescent as is that of *U. nuda.* Certain heads altho loose in appearance possess distinctly compact spore masses. The spore wall is echinulate as in *U. nuda.* The echinulations are of variable distinctness in different collections. Seedling infection thru seed- and secondary flower-inoculation, and control by seed treatment (dusting) have been observed.

Type 5.—This type differs from Type 4 only in the paleness of its spores and differs from Type 5 (Ruttle, 1933). The sori are loose, the spore wall distinctly echinulate and the spore mass *light olivaceous brown* as in *U. nuda* but the spores germinate by promycelia as in *U. hordei.* The material was collected on Featherston barley both in the field and greenhouse. Inoculations should be made to determine whether its progeny will be equally pale-spored.

Type 6.—This type was described as Type 5 by Ruttle (1933). The mode of branching and septation of the germ tubes are not now regarded as sufficiently distinctive to be used for type separation.

FIG. 1.—TYPICAL SMUTTED HEADS INFECTED WITH TYPES 1 TO 7.

Numbered in order, from left to right. *U. hordei* (Type 1), Types 2, 3, and 4, and *U. nuda* (Type 7) are on Alpha and Types 5 and 6 on Featherston barley. ⅔ natural size.

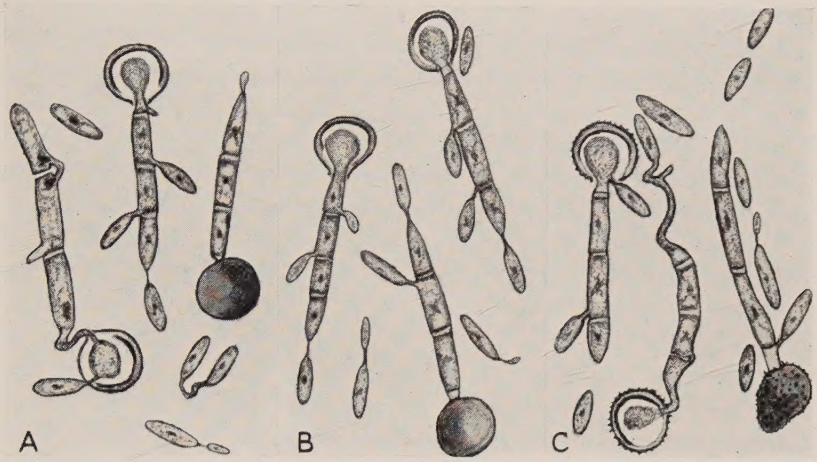

FIG. 2.—CAMERA LUCIDA DRAWINGS OF SPORES OF (A) *U. hordei*, (B), TYPE 3, AND (C) TYPE 4.

24 hours after being put to germinate at 20° C. x 1120.

It is now distinguished from *U. nuda* only by the fact that the spore masses are considerably darker than those of typical *U. nuda* and of Type 5 and not separable in color from that in paler heads of Type 4.

Type 4 occurs frequently in fields near Geneva, N. Y. It was also collected near Maidstone, Ontario, and received from Dr. Tapke. Types 2, 3, 5, and 6 were collected only occasionally. The variations noted in Type 4 may also occur in these types were sufficient material available for examination.

Type 3 corresponds closely with the figures and description of "charbon nu tardif" described by Vanderwalle (1932) which has temporarily covered sori in spikele's and smooth or slightly reticulate spores. The lateness in development of the smutted heads which gave the designation "tardif" to Vanderwalle's new loose smut was not recorded, if present, for Type 3. Vanderwalle regards this smut as a hybrid between *U. nuda* and *U. hordei*. There is, however, some question whether the *U. nuda* he used may not have been Type 4 since he obtained seedling infection with it by Tisdale's (1923) method.

The spore wall of *U. nigra* is not described by Tapke (1932) so that it may well include both Type 3 and the darker-spored variants of Type 4, this especially since plants grown from seed "flower-

inoculated" with material received from Dr. Tapke were infected with both these types altho Type 4 predominated. The original material received from Dr. Tapke may have been an admixture of the two types altho only echinulate spores were observed. Material at Cornell identified by Dr. Tapke as *U. nigra* was echinulate-spored. It, consequently, seems probable that *U. nigra* applies particularly to the darker-spored variants of Type 4.

Biedenkopf's description of *U. medians* does not mention spore color, so that *U. medians* could include Types 4 and 5 but was probably applied to Type 4, this type appearing to be the more prevalent.

Accordingly, the name *U. medians* should probably supplant the name *U. nigra* and the type number provided that on further investigation the fungus is found to deserve species designation. The writer has for this paper retained the type number, since in inoculation studies Types 3, 4, and 5 have been obtained from material which to all appearances was typical Type 4 and since some indications of hybridization between Type 3 and *U. nuda* have been obtained.

GREENHOUSE AND FIELD STUDIES

MATERIAL AND METHODS

Three varieties of barley, Alpha, Featherston, and Tennessee Winter, and two varieties of wheat, Reward and Honor, were selected for experiment.

For inoculation purposes the following collections of smut from the same field were used: *U. hordei,* consisting of several infected heads; Type 2, one head; Type 3, one head; Type 4 (field collection), several infected heads; and Type 4, consisting of one head No. 44. In addition the following were used: Material furnished by Dr. Tapke and called in this paper Type 4 (Tapke collection); Type 6 collected in a second field; *U. nuda* collected on Featherston barley in the greenhouse; *U. tritici,* collected on Honor wheat grown in the greenhouse at Geneva and called *U. tritici* (Honor form); and *U. tritici* collected on Garnet wheat in Manitoba by Dr. Hanna and called *U. tritici* (Reward form).

Flowers of the three above-named varieties of barley and of the two varieties of wheat were inoculated with *U. nuda* (Featherston collection); flowers of the three varieties of barley and of Reward

wheat with Type 4 (Tapke collection); flowers of Honor and Reward wheat and of Alpha and Featherston barley with *U. tritici* (Reward form); and flowers of Honor and Reward wheat with *U. tritici* (Honor form).[3]

All flower-inoculations were performed in the greenhouse by the common method of opening the glumes with a small forceps and dusting the floral parts with a small camel's hair brush which had been dipped in spores. Flowers were used on which the glumes at the time of inoculation would open within approximately 24 hours, were open, or had closed within 24 hours.

Seed was inoculated for both field and greenhouse experiments by the following methods:

1. Hulled or dehulled seed was rolled in spores just previous to sowing in the greenhouse. In all cases dehulling was by hand (Tapke, 1923).

2. Hulled or dehulled seed was rolled in spores and put to germinate at room temperature on potato dextrose agar for 2 to 5 days before sowing.

3. Barley seed and spores of Type 4 (field collection) were placed in a filtering flask which contained 1½ per cent malt solution. The flask was corked securely, evacuated for 10 minutes, the air allowed to re-enter, and the flask again evacuated, after which the seed was spread on a paper towel for ½ to 2 hours to dry, then sown in the field. The procedure permitted the spores to penetrate between the glumes.

4. A sporidial suspension of Type 4 was used instead of spores, otherwise the treatment was the same as in 3. Both 3 and 4 are modified from the methods of Haarring (1930), and others for effectively inoculating oats with *Ustilago avenae*.

GREENHOUSE AND FIELD RESULTS IN 1932 AND 1933

U. hordei.—In the greenhouse all three varieties of barley were infected when inoculated by methods 1 and 2, with spores of *U. hordei*. Proportionally more plants from dehulled than from hulled seed were infected. Reward wheat was not infected.

Type 2.—In the greenhouse seed of Alpha and Featherston barley inoculated with spores of Type 2 by methods 1 or 2 produced plants infected with this same type of smut (Table 2, 153, 154; Fig. 1).

[3] For the sake of brevity, seed from flowers that had been inoculated is sometimes spoken of as "flower-inoculated" seed.

Type 3.—Featherston barley grown from seed inoculated by methods 1 or 2 with spores of Type 3 was infected with Type 3. Alpha barley similarly inoculated was infected with *U. hordei,* that is, the spores were smooth as in Type 3 but the sori were covered by a definite membrane as in *U. hordei.* A few heads infected with Type 3 appeared in Alpha barley grown from seed "flower-inoculated" with Type 4 (Tapke collection). Alpha inoculated with this material was infected with typical Type 3 (see hybridization, 2b29).

Type 4 and *Type 5* (*collection No. 44*).—Seed of Alpha and Featherston barley was pretreated with hot water to rid it of smut, then inoculated by methods 1 and 2 with spores from collection No. 44, which appeared to be typical Type 4. Smutted plants were produced, the majority of which were infected with either Type 3 or Type 4 or an occasional head with a mixture of both types 3 and 4. However, one head of one Alpha plant which was largely infected with Type 3 contained a small pale-spored area of Type 5. Also, one out of two heads on a Featherston plant was infected with Type 5 and the second head with a mixture of Types 3 and 4. Altho considerable color variation regularly occurs in material regarded as Type 4, in no other case were spores found which microscopically or in mass were so pale as in these Type 5 heads, and which, at the same time, germinated by promycelia and sporidia. Because of the extreme paleness of the spores, the material has been tentatively called Type 5 to distinguish it from the darker-spored Type 4. The progeny should be investigated. Type 5 was also collected on Featherston barley in the field.

Type 4 (*field collection*).—Spores of this collection produced no infection by seed-inoculation on seven Featherston plants grown to maturity but produced 13 per cent infection on the Alpha plants. All smutted heads were infected with Type 4. (Table 2, 156–163.)

Type 4 (*Tapke collection*).—Alpha seed from flowers inoculated with Type 4 (Tapke collection) gave 45 per cent smutted plants (Table 2, 1–2), Tennessee Winter gave 7 per cent, while Featherston gave none at all. Of the infected Alpha plants, the majority of smutted heads bore echinulate spores medium to dark brown in mass (Type 4). A few heads bore smooth spores or the major part smooth and an occasional one slightly echinulate and in mass somewhat darker brown that those of Type 4 (Type 3). At times early heads of Alpha plants escaped infection and later heads were infected. The seedling distortion described by Tisdale and Tapke (1924) was noted, but no counts were kept of the mortality.

Control in the greenhouse was obtained by dusting the "flower-inoculated" seed with Semesan, Ceresan, Improved Ceresan, or red copper oxide (Horsfall, 1932) previous to sowing. No or very little infection was obtained when the inoculated seed was germinated and grown in a very hot greenhouse.

Type 6.—Neither seed nor flower inoculation with Type 6 led to infection due to the much-overheated condition of the greenhouse at the time of seed germination.

U. nuda.—Seed of Alpha and Featherston barleys from flowers inoculated with spores of *U. nuda* was sown in March and seed of Tennessee Winter similarly inoculated was sown in February. As shown in Table 2, 64 per cent of Alpha and 85 per cent of Featherston plants were infected. Similarly inoculated seed of Featherston barley gave no infection when sown on June 1 in a much over-heated greenhouse. The fungus was not at all controlled by the application of dust treatments before sowing but was controlled by the hot water treatment. Tennessee Winter was not infected by *U. nuda.*

Other smuts.—Reward wheat was not infected by rolling the seed in spores of *U. hordei* or Type 4 before sowing nor by flower-inoculation with *U. nuda,* Type 4, or *U. tritici* (Honor form). Flower-inoculation under the same conditions with *U. tritici* (Reward form) produced 100 per cent infection. Flower-inoculation of Honor wheat with *U. tritici* (Reward form) produced no infection, whereas similar inoculation with *U. tritici* (Honor form) produced 86 per cent infection (Table 2). Dusting the seed with Semesan, Ceresan, or red copper oxide previous to sowing did not lessen the amount of *U. tritici.*

The results from field plats in 1933 for the most part corroborated the greenhouse results listed above. Untreated seed of both Alpha and Featherston barley from plats planted with Semasan-dusted seed in 1932 gave only a small percentage of infection, all of which was typical loose smut (*U. nuda*). Dusting of this seed with Semesan, Ceresan, or red copper oxide before sowing did not diminish the amount of smut present.

Untreated seed of Alpha barley from a field heavily smutted in 1932 showed approximately 20 per cent smutted heads belonging to *U. hordei,* to intermediate Types 2, 3, and 4, and to *U. nuda.* When this seed was dusted with Semesan or Ceresan before sowing, the amount of infection in early counts was reduced to less than 1 per cent belonging to *U. nuda, U. hordei,* and Type 4.

TABLE 2.— RESULTS FROM INOCULATED SEEDS GROWN IN THE GREENHOUSE IN 1932 AND 1933.

SMUT	GREENHOUSE NO.	VARIETY	METHOD OF INOCULATION	SEED TREATMENT	NUMBER OF PLANTS		TYPE OF SMUT
					Infected	Noninfected	
U. hordei (No. 160)	184–8	Alpha	Seed, 1 and 2	None	17	39	*U. hordei;* occasional spores echinulate, others smooth
	189–92	Alpha	Seed, 2	Hot water pretreated	1	2	*U. hordei;* occasional spores echinulate, others smooth
	182–3 195–6	Featherston	Seed, 2	Hot water pretreated	1	32	*U. hordei;* occasional spores echinulate, others smooth
	193–4 197–8	Tenn. Winter	Seed, 2	None	8	46	*U. hordei;* occasional spores echinulate, others smooth
	172–3	Reward	Seed, 2	None	0	15	No infection
Type 2	153	Alpha	Seed, 1 and 2	None	3	0	Type 2
	154–5	Featherston	Seed, 1 and 2	Hot water pretreated	1	18	Type 2
Type 3 (No. 109)	178–9	Alpha	Seed, 1 and 2	Hot water pretreated	1	17	*U. hordei;* occasional spores echinulate, others smooth
	180–1	Featherston	Seed, 1 and 2	Hot water pretreated	1	10	Type 3
Type 4 (coll. 44)	140–1	Alpha	Seed, 1	None	8	8	Type 4
	143–6	Alpha	Seed, 1 and 2	Hot water pretreated	8	24	Types 3, 4, and 5
	147–5	Featherston	Seed, 1 and 2	Hot water pretreated	6	35	Types 3, 4, and 5
Type 4 (field coll.)	156–9	Alpha	Seed, 1 and 2	None	2	20	Type 4
	160–1	Alpha	Seed, 1	Hot water pretreated	1	0	Type 4
	162–3	Featherston	Seed, 1	Hot water pretreated	0	7	No infection
	171, 177	Reward	Seed, 1	None	0	38	No infection
Type 4 (Tapke Coll.)	1–2	Alpha, March seeding	Flower (1932)	None	37	46	Types 3 and 4; echinulations more or less marked on spores from different heads
	235–6 250–1	Alpha, June seeding	Flower (1932)	Hot green house	2	57	Type 4
	3–8	Alpha, March seeding	Flower (1932)	Semesan, Ceresan, Cu_2O	0	36	No infection
	11–2	Featherston	Flower (1932)	None	0	48	No infection
	13–8	Featherston	Flower (1932)	Semesan, Ceresan, Cu_2O	0	31	No infection
	21	Tenn. Winter	Flower (1932)	None	3	41	Type 4
	22–8	Tenn. Winter	Flower (1932)	Semesan, Ceresan, Cu_2O	0	41	No infection
	31–2	Reward	Flower (1932)	Dusted and not dusted	0	2	No infection
Type 6	168	Alpha, June seeding	Seed, 1 and 2	None, hot green house	0	2	No infection
	169–70	Featherston, June seeding	Seed, 1 and 2	Hot water, hot green house	0	18	No infection
	223–4	Featherston, June seeding	Flower (1933)	Hot green house	0	67	No infection
U. nuda	202, 204	Alpha	Seed, 1	Hot water pretreated	0	4	No infection
	207, 211	Tenn. Winter	Seed, 1	Hot water pretreated	0	19	No infection
	41–2	Alpha, March seeding	Flower (1932)	None	62	35	*U. nuda;* average 64% infection
	43–8	Alpha, March seeding	Flower (1932)	Semesan, Ceresan, Cu_2O	12	9	
	49–50	Alpha, March	Flower (1932)	Hot water	0	20	No infection
	51–2	Featherston	Flower (1932)	None	39	11	*U. nuda;* average 85% infection
		Featherston	Flower (1932)	None	75	10	
	53	Featherston	Flower (1932)	Semesan, Ceresan, Cu_2O	22	4	

TABLE 2.— *Concluded.*

SMUT	GREENHOUSE NO.	VARIETY	METHOD OF INOCULATION	SEED TREATMENT	NUMBER OF PLANTS		TYPE OF SMUT
					Infected	Non-infected	
U. nuda	59–60	Featherston	Flower (1932)	Hot water	0	18	No infection
	223–4	Featherston, June, seeding	Flower (1932)	Dusted and not dusted	0	64	No infection
	61–2	Tenn. Winter	Flower (1932)	None	0	54	No infection
	63–8	Tenn. Winter	Flower (1932)	Dusted	0	67	No infection
	69–70	Tenn. Winter	Flower (1932)	Hot water	0	16	No infection
	71	Reward	Flower (1932)	None	0	87	No infection
	72	Reward	Flower (1932)	Dusted	0	12	No infection
U. tritici (Reward form)	75–6	Alpha	Flower (1932)	None	0	31	No infection
	77–8	Alpha	Flower (1932)	Ceresan	0	14	No infection
	81–2	Featherston	Flower (1932)	None	0	55	No infection
	83–4	Featherston	Flower (1932)	Ceresan	0	9	No infection
	86	Reward	Flower (1932)	None	16	0	*U. tritici;* 100% infection
	87–8	Reward	Flower (1932)	Ceresan,Cu_2O	15	0	*U. tritici;* 100% infection
	93	Honor	Flower (1932)	None	0	11	No infection
	94–5	Honor	Flower (1932)	Ceresan,Cu_2O	0	26	No infection
U. tritici (Honor form)	90	Reward	Flower (1932)	None	0	19	No infection
	91–2	Reward	Flower (1932)	Ceresan,Cu_2O	0	20	No infection
	98	Honor	Flower (1932)	None	26	1	*U. tritici;* average infection 86%
	99–100	Honor	Flower (1932)	Ceresan,Cu_2O	11	8	
No inoculation	101–08	Alpha	None	None	1	211	108*b*, Type 4 and *U. nuda*
	111–16	Featherston	None	Semesan, Ceresan	0	54	No infection
	122–23	Tenn. Winter	None	Semesan, Ceresan	0	88	No infection
	133–35	Reward	None	Semesan, Ceresan	0	28	No infection
	129–31	Honor	None	Semesan, Ceresan	0	27	No infection

Featherston barley obtained from a smutted field was also dusted with Semesan, and Ceresan. The proportion of *U. nuda* in this seed was very high, so that dusting reduced the amount of smut by only one-half. The majority of the smutted heads examined, were *U. nuda* but two were of Type 4 and two of Type 6.

Field results from Alpha and Featherston seed from flowers inoculated with spores of Type 4 (Tapke collection) corroborated the greenhouse results. Alpha gave approximately 50 per cent infection with Type 4 (Type 3 also present) when sown without treatment, while only a few late-smutted heads were found when the seed was dusted with Semesan and considerably more late heads when dusted with red copper oxide. There were no infected Featherston plants even when the seed was untreated.

Uninoculated certified seed of Alpha barley gave no infected plants with or without treatment. When this seed was inoculated with spores of *U. hordei* by method 1, 7 per cent of infected heads

were obtained; but when inoculated by method 3, the amount of infected heads increased to 17 per cent. When inoculated with Type 4 by methods 1, 3, and 4, considerable percentages of smut were obtained, particularly by method 4.

Under the conditions of these experiments there is a distinct difference in percentage of infection depending on whether infected heads or infected plants are counted, infected plants having as a rule fewer heads than uninfected plants or being only partially infected. Thus, in one plat only 17 per cent of the heads were infected, while 30 per cent of the plants were infected.

SUMMARY AND DISCUSSION OF GREENHOUSE AND FIELD RESULTS

By seed inoculation, the collection of *U. hordei* used produced infection in the greenhouse on all three barley varieties; the collections of Types 2, 3, and 4 (collection No. 44) produced infection on both Alpha and Featherston barley; and Type 4 (field collection) produced infection on Alpha but not on Featherston. By flower-inoculation, Type 4 (Tapke collection) produced infection on Alpha and Tennessee Winter barley but not on Featherston, which seemed resistant to this particular collection, while *U. nuda* produced infection on Alpha and Featherston but not on Tennessee Winter barley.

Type 4 (Tapke collection) yielded on inoculation Types 3 and 4, Type 4 (collection No. 44) yielded Types 3, 4, and 5; and Type 4 (Field collection) yielded only Type 4.

Dehulling seed flower-inoculated with Type 4 before planting did not diminish the amount of Type 4. Dehulling uninoculated seed increased the amount of Type 4 and *U. hordei* obtained by seed inoculation.

Of the two forms of *U. tritici* used, the Reward form is most probably that described by Hanna and Popp (1932) as infecting many of the common wheats in Manitoba, while the Honor form may be the equivalent of their second form or may be still a third form. Grevel (1930) reports four physiologic forms of *U. tritici*, one of which may correspond to the Honor form.

The resistance of Tennessee Winter barley to the Featherston form of *U. nuda* agrees with the findings of Nahmmacher (1932) who recognized two forms of *U. nuda* as occurring in Germany, the one on spring barleys, the other on winter barleys.

In the greenhouse dusting "flower-inoculated" seed before planting with Semesan, Ceresan, or red copper oxide did not decrease

the amount of *U. tritici* or *U. nuda* but effectually controlled Type 4. In the field plats, *U. nuda* was controlled by hot water treatment only and not at all by dusting before planting with Semesan or Ceresan which gave considerable control of *U. hordei* and Types 2, 3, and 4.

The control of *U. nuda* by treating the seed with formaldehyde, copper carbonate, and various mercury compounds reported by Johnson (1914), Tisdale, *et al.* (1923, 1924, and 1927), and others is explained by the failure to distinguish between the true blossom-infecting loose smut and the seedling-infecting loose smuts, Types 3 and 4, which, in the case of the darker-colored heads, superficially resemble *U. nuda* and in the case of the lighter-colored heads of Type 4 and of mixed heads are impossible to distinguish except by germination tests.

HYBRIDIZATION

MICROMANIPULATOR

A micromanipulator, being essential for the isolation of sporidia and none being available, was devised in the following manner. The binocular tube was removed from a Leitz research microscope. The mechanical stage (Fig. 3, 1) was attached to a wooden bar (Fig. 3, 2) which in turn was attached to a lead bar (Fig. 3, 3) filed to fit the tube groove and clamped into position by the tube clamp. In this way the coarse and fine adjustments of the microscope were used to raise and lower the mechanical stage, the stage itself giving movement forward and back and right and left.

An arm about 7 inches long (Fig. 3, 4), consisting of six layers of lead, extended from the mechanical stage to the stage level of the visual microscope. This arm was attached to a brass bar (Fig. 3, 5) which was filed to fit the groove in the mechanical stage which carries the slide-holding bar.[4]

The lead layers are held firmly by three brass rings, in the lower one of which there is a hole and support for a compass needle holder (Fig. 3, 6) which in turn holds a steel needle (Fig. 3, 7) with a bent tip for picking off sporidia. Plasticine and hard rubber are used to hold the compass needle holder more firmly. The lead arm, the

[4] Dr. Kearsley and Mr. C. P. Haskins of the General Electric Co., Schenectady, N. Y., and Mr. Karl Brase of this Station assisted in fitting and devising parts of the micromanipulator.

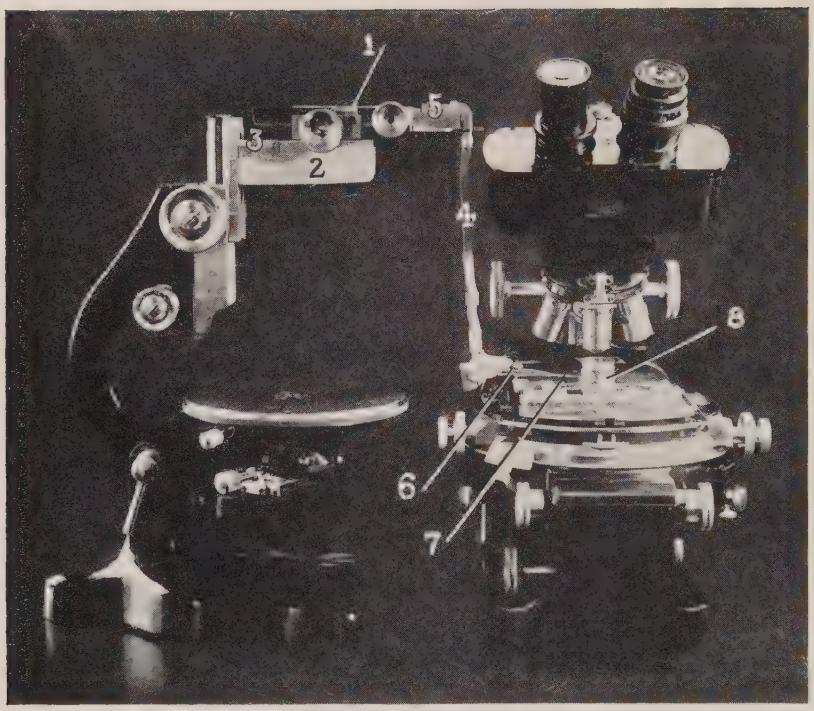

Fig. 3.—Micromanipulator at the Left of the Visual Microscope. Description in text.

plasticine for supporting the compass needle holder, and a glyptol pad beneath the miscroscope all serve to absorb vibration so that the needle is comparatively steady.

The manipulator microscope was placed to the left of the visual microscope, the stage of the former serving as a hand rest in the manipulation of the screws.

A suitable glass cell (Fig. 3, 8) easy to sterilize was made by attaching by means of a resinous mixture three glass micro-slide rings 5 mm high and 18 mm in diameter to each other and to a slide. A part was removed from the side of the two upper rings so that the needle could be manipulated easily within the cell. The lower ring was left intact and held a slight amount of water which prevented drying out of cultures while working (modified from Dickinson, 1926).

MONOSPORIDIAL CULTURES AND INOCULATION

In order to observe the relationships between the intermediate types of barley smut and *U. nuda* and *U. hordei* attempts were made to cross Type 3 and *U. hordei,* Type 3 and *U. nuda* and *U. hordei* and *U. nuda.* For this purpose nine monosporidial cultures were made, three of Type 3, plant 2b29,[5] spore 3 (2b29.3.1, 3.2, 3.3) and six of *U. hordei,* plant 185, spores 1, 2, 4, and 6 (185.1.1, 2.1, 2.4, 4.1, and 6.4). The methods for culturing spores, for isolating single sporidia, and for making monosporidial cultures described by Hanna (1928) have been followed thruout with slight modifications. Monosporidial cultures were numbered according to the method of Hanna (1929) except that the number of the sporidium does not apply in every case to the number of the promycelial cell from which it developed. No cultures of *U. nuda* were made which can be regarded as the equivalent of the monosporidial cultures of *U. hordei* and Type 3 altho the method of Dickinson (1933) makes such cultures possible.

Fusion studies between sporidia from the nine monosporidial cultures showed that they fall into two sex groups. To the one group belongs cultures 2b29.3.2, 185.2.1, 185.2.4, 185.4.1, and 185.6.4 and to the other cultures 2b29.3.3, 185.1.1 and 185.4.2 (Table 3). On weakly alkaline medium fusions occurred between sporidia of opposite sex groups but never between sporidia within a given group. Sporidium, 2b29.3.1, from both fusion and inoculation studies is evidently not a monosporidial culture since fusions occur between it and members of both groups and since it produces on inoculation a pure Type 3 (Table 4). It is included since it was used in inoculations before its impurity was recognized.

Inoculations of barley seed with the various cultures and their combinations were made during very hot weather in late July. Since it was recognized that intense heat in the greenhouse at the time of sowing prevents infection, an attempt was made to offset this effect by incubating the inoculated seed for 4 days at a temperature of 20° C which Faris (1924, B) and others have found favored infection. The seed to be inoculated was placed on moist filter paper in a sterile petri dish, the inoculum growing on potato dextrose agar or in malt solution added to the seed which was then incubated. On the second day many of the coleoptiles were crushed in the inoculum

[5] Collection 2b29 was from one of 37 infected plants grown in the greenhouse from seed flower-inoculated with Type 4 (Tapke collection).

TABLE 3.— SPORIDIAL FUSIONS BETWEEN SIX MONOSPORIDIAL LINES OF *U. hordei* AND THREE OF TYPE 3.*

		U. hordei, PLANT 185						TYPE 3, PLANT 2b29		
		1.1	2.1	2.4	4.1	4.2	6.4	3.1	3.2	3.3
U. hordei, PLANT 185	1.1	—	+	+	+	—	+	+	+	—
	2.1	+	—	—	—	+	—		—	+
	2.4	+	—	—	—	+	—		—	+
	4.1	+	—	—	—	+	—		—	+
	4.2	—	+	+	+	—	+		+	—
	6.4	+	—	—	—	+	—		—	+
TYPE 3, PLANT 2b29	3.1		+	+	+					+
	3.2	+	—	—	—	+	—	+	—	+
	3.3	—	+	+	+	—	+	+	+	—

* The first number in the column is the spore number, the second the sporidium number. Plus signs indicate fusions, minus signs lack of fusions. Culture 2b29.3.1 is evidently not a monosporidial culture.

in the hope that injury might favor infection. Too great moisture in the petri dishes had to be avoided since it prevented seed germination and possibly infection. Even with these precautions infection was erratic (Table 4), being in certain cases good, in many considerably less than was to have been expected and in others where there should have been infection it was entirely lacking. No account was taken of acidity altho this possibly was of importance (Reed and Faris, 1924).

RESULTS OF INOCULATIONS

Inoculations of Alpha barley with monosporidial cultures of *U. hordei* (185.1.1, 185.2.1, and 185.2.4) or of Type 3 (2b29.3.2, and 2b29.3.3) gave negative results (Table 4, 266-268, 264b, 265).

When two monosporidial cultures of opposite sex of *U. hordei* (185.2.1 and 185.1.1; and 185.2.4 and 185.1.1) were used to inoculate, the sori in the smutted heads were typically covered and bore smooth spores. Positive results were not obtained by inoculating seed with two monosporidial cultures of Type 3 (2b29.3.2 and 3.3) of opposite sex, but the smutted heads obtained from inoculating with 2b29.3.1 alone and with 2b29.3.1 and 3.2 are all typical loose Type 3 heads bearing smooth spores.

The F_1 progeny (Table 4, Nos. 271b, 272b, and 301) from a cross between a monosporidial culture of *U. hordei* and one of Type 3 of opposite sex (185.2.1 x 2b29.3.3 and 185.2.4 x 2b29.3.3) on both Alpha and Featherston barley is indistinguishable from Type 3, except that the sori are possibly somewhat more covered (Fig. 4).

FIG. 4.—SMUTTED HEADS PRODUCED BY INOCULATING SEED WITH TWO MONOSPORIDIAL CULTURES OF OPPOSITE SEX.

1 and 2, *U. hordei*. 1, culture 185.2.4 x 185.1.1. 2, culture 185.2.1. x 185.1.1. 3 and 4, $_1$F *U. hordei* x Type 3. 3, culture 185.2.4 x 2b29.3.3; 4, culture 185.2.1 x 2b29.3.3. 5, Type 3, culture 2b29.3.1 x 2b29.3. 6, Type 3 head from a plant other heads of which were infected with typical *U. nuda*. ⅔ natural size.

When a multispore or monospore culture of *U. nuda* was used to inoculate together with a monosporidial culture of *U. hordei* or Type 3, no infection was obtained with the exception of inoculation with culture 2b29.3.1, which is evidently not monosporidial and which gave typical Type 3 heads. When a multisporidial culture of Type 4 was used together with a multispore culture of *U. nuda,* only typical Type 4 was obtained.

Attempts were also made to secure hybrids between *U. hordei* and *U. nuda* and between Type 3 and *U. nuda* by using seed from flowers inoculated with *U. nuda* for inoculation with *U. hordei* or Type 3, respectively.

TABLE 4.—RESULTS OF INOCULATIONS WITH CULTURES ON ALPHA AND FEATHERSTON BARLEY.

INOCULATION WITH			GREENHOUSE No.	NUMBER OF PLANTS		TYPE OF SMUT
U. nuda	*U. hordei*	Type 3, 2b29		Infected	Non-infected	
			ALPHA			
Flower +	183*a*, spores applied	.....	254	12	4	9 plants *nuda*, 1 *hordei*, 2 *hordei* and *nuda* on separate heads of same plant
Flower +	185 spore 1 sporid. cult.	.....	257	7	16	*U. nuda*
Flower +	185.2.1.....	.....	259	8	3	7 plants *nuda*, 1 *nuda* and F_1 (?) in same head
Flower +	.....	Spore 5, sporid. cult.	255	6	1	5 plants *nuda*, 1 *nuda* and Type 3 on separate heads of same plant
Flower +	.....	2b29.3.1 + 2b29.3.2	256	15	5	11 plants *nuda*,1 Type 3, 3 *nuda* and Type 3 in same head and 2 heads *nuda*, Type 3 and F_1
Flower +	.....	2b29.3.2	258*a*	4	2	*U. nuda*
Flower +	.....	2b29.3.1	258*b*	11	1	10 plants *nuda*, 1 *nuda* and Type 3 in same head
Multispore culture +	.....	2b29.3.1	260 and 290	6	68	Type 3
Spores of 42+	.....	3.3	291	0	38	No infection
Monospore culture 56 (1)+	.....	3.3	276	0	27	No infection
Monospore culture of 108+	.....	Spore 2, sporid. cult.	278	0	25	No infection
Monospore culture of 108+	.....	3.2	281	0	37	No infection
Multispore culture+	.....	Type 4, multispore culture	285	2	30	Type 4
Monospore culture 56 (1)+	185.2.1	.....	282	0	34	No infection
Multispore culture+	185.1.1	.....	283	0	23	No infection
Monospore culture 42	.....	.....	279	0	28	No infection
Monospore culture of 108	.....	.....	280	0	27	No infection
.....	187, spores applied	.....	289	4	23	*U. hordei*
.....	Multisporidial culture	.....	295	11	35	*U. hordei*
.....	Spores, by evacuation	.....	294	3	45	*U. hordei*
.....	185.1.1	.....	266	0	28	No infection
.....	185.2.1	.....	267	0	67	No infection
.....	185.2.4	.....	268	0	20	No infection
.....	2.1+1.1	.....	273*a*	6	26	*U. hordei*
.....	2.4+1.1	.....	273*b*	7	29	*U. hordei*
.....	.....	3.1+3.2	264*a*	12	18	Type 3
.....	.....	3.3+3.2	240 and 288	0	55	No infection
.....	.....	3.1	263	0	23	No infection
.....	.....	3.2	264*b*	0	28	No infection
.....	.....	3.3	265	0	27	No infection
.....	185.2.1+	3.3	271*b*	8	33	F_1 *U. hordei* x Type 3; very similar to Type 3
.....	185.2.4+	3.3	272*b*	4	12	F_1 *U. hordei* x Type 3; very similar to Type 3

TABLE 4.— *Concluded.*

Inoculation with			Greenhouse No.	Numbers of plants		Type of smut
U. nuda	*U. hordei*	Type 3, 2b29		Infected	Non-infected	
			FEATHERSTON			
Flower +	183*a*, spores	.....	287	10	4	*U. nuda*
Flower +	185.2.4	.....	286	3	6	*U. nuda*
.....	.....	Spore 5, sporid. culture	300	0	39	No infection
.....	185.2.4+	3.3	301	2	30	F_1 *U. hordei* x Type 3

A monosporidial culture of Type 3 (2b29.3.2) used to inoculate Alpha seed "flower-inoculated" with *U. nuda* produced no apparent effect, all infected heads being typical *U. nuda.* When a monosporidial culture 185.2.1 of *U. hordei* was used to inoculate Alpha seed from flowers inoculated with *U. nuda* and 185.2.4, used to inoculate Featherston seed similarly inoculated with *U. nuda,* all smutted heads were affected with typical *U. nuda* except a single head of one Alpha plant. This head was badly infected with bacteria and mould and in consequence most spores did not germinate, altho a few germinated slowly by germ tubes and occasionally one by promycelia and sporidia.

When a multisporidial culture of *U. hordei* (Table 4, greenhouse No. 254) or Type 3 (Table 4, No. 256) was used in inoculating Alpha seeds from flowers inoculated with *U. nuda,* most plants were infected with *U. nuda* only and a few plants with *U. hordei* or Type 3 only. Other plants bore some heads infected with *U. hordei* (Table 4, No. 254) or Type 3 (No. 255) and other heads infected with *U. nuda,* while still other plants bore heads containing spores of both Type 3 and *U. nuda* (No. 256) in the same head. The majority of these mixed heads were, except for the somewhat darker spore mass, typical *U. nuda* in appearance. They could be distinguished only by examining the germinating spores under the microscope, smooth spores germinating by promycelia and sporidia (Type 3), echinulate spores by germ tubes (*U.* nuda).

One smutted head of plant 10 (No. 256) resembled closely the other two of this plant which were infected with *U. nuda* only, except that the spores in mass were considerably darker. The spores of this darker head were *echinulate,* and while most of them germinated by germ tubes (Fig. 5, B), a few germinated by promy-

FIG. 5.—CAMERA LUCIDA DRAWINGS OF SPORES OF TYPE 3 x *U. nuda* (?) (A) AND OF *U. nuda* (B).
36 hours after being put to germinate at 26° C. x 1120.

celia which either cut off sporidia normally Fig. 5, A), as in Type 4 (Fig. 2, C), or the sporidial initiate grew out into a branch which cut off sporidia at the end (Fig. 5, A). Some sporidia were normal appearing, while others were less oval and more elongate spindle-shaped than normal, a type which was observed to develop only from echinulate spores of plant 19 (No. 256). The single smutted head of this plant was not typical, since it was infected with bacteria and a fungus as well as with three types of smut spores, *viz.*, smooth, germinating by promycelia which cut off sporidia (Fig. 2, B, Type 3); echinulate, germinating by germ tubes (Fig. 5, B, *U. nuda*); and echinulate spores, germinating by promycelia which cut off sporidia (Fig. 5, A). The origin of these echinulate chlamydospores which germinate by promycelia which cut off sporidia can be regarded as

resulting from the hybridizing of *U. nuda* and Type 3 or from the segregating or mutating of Type 3 or *U. nuda*. No trace of segregation or mutation was observed in other Type 3 or *U. nuda* cultures.

DISCUSSION AND SUMMARY

The existence of a smut (Type 4, *U. medians, U. nigra*) intermediate between *U. nuda* and *U. hordei* tempts speculation, backed by experiment, as to its possible hybrid origin, especially since so many smut species are at present being hybridized and since so many types intermediate between *U. nuda* and *U. hordei* exist of which, to be sure, the more common lie between Type 4 and *U. hordei*. The possibility of hybridization between *U. nuda* and *U. hordei* seems the more probable, Kniep (1926) having found that sporidia of *U. hordei* readily copulate with individual cells of the germ tubes of *U. nuda*. Buller (1930) has also shown that a diploid mycelium of *Coprinus* may "diploidize" a haploid mycelium of the same species. Buller (1931 and earlier), Dickinson (1932), and earlier writers have shown that diploid hyphae of the same species or different forms of the same species readily fuse in culture. From these results it seems that, if *U. nuda* and *U hordei* are capable of fusion outside of the host (Kniep, 1926), fusion and consequent hybridization might well occur between hyphae growing in close proximity within the host, which is the more likely place of successful fusion owing to the different infection capabilities of the two fungi. If, as evidence indicates, the *echinulate spores germinating by promycelia and sporidia* in smutted heads of plants from seed which had been "flower-inoculated" with *U. nuda* and "seed-inoculated" with Type 3 are F_1 chlamydospores, hybridization must have occurred by fusions between diploid mycelium at some point in the growth of the host plant.

Results indicate that Type 4 may have arisen by hybridization, but it is also possible that it originated as a mutant from *U. hordei* or *U. nuda* or that they came from Type 4, in which case Types 2 and 3 and their variants may be regarded as hybrids, hybrid segregants, and back crosses of the cross *U. hordei* x Type 4, and Types 5 and 6 and their variants as possibly hybrids, hybrid segregants and back crosses of the cross Type 4 x *U. nuda*. Or Type 5 may be regarded as a pale-spored mutant of Type 4 and Type 6 as a dark-spored mutant of *U. nuda*.

Mutations have been found to occur in the rusts (Newton and Johnson, 1927; and others). In the smuts the wheat disease recorded by Campagna (1925) as having all the morphological characteristics of *U. tritici* except that the spores were smooth, colorless, and nearly transparent (no germination or infection studies were recorded) may have been a mutant of *U. tritici.*

Type of germination, while having been regarded as sufficiently stable to use as a basis for species separation, has at the same time been found by numerous workers to be greatly influenced by temperature and by media. Hüttig (1931) showed that, in nine species of *Ustilago,* the temperature at germination influenced decidedly the type of germination, *U. tritici* germinating by promycelia and sporidia below 10° C, and *U. hordei* and *U. avenae* by germ tubes at 30° C, and *Ustilago longissima* by promycelia and sporidia at 35° C. Such considerations tend to show that *U. hordei* and *U. nuda* may possibly be less far removed from each other than type of germination at 20° C has indicated.

Diehl (1925) observed that spores of *U. avenae* applied to oat stigmas germinate in moist cool weather by promycelia and sporidia and in warm dry weather by germ tubes. Kolk (1930) in one case figures the infecting hyphae of *U. avenae* entering the host directly from the spore. Tisdale and Tapke (1924), in examining barley seedlings grown in sterile test tubes and inoculated with a seedling-infecting *U. nuda* (probably Type 4), record no sporidia but were able to trace the infecting hyphae directly to the chlamydospores. This may have been due either to the temperature or to the conditions of moisture and may account for their failure to recognize sporidial formation.

In summary, monosporidial cultures of *U. hordei* or Type 3 on inoculation produce no infection, bisporidial cultures of opposite sex produce infection in type similar to that of the parent. The F_1 between Type 3 and *U. hordei* resembles Type 3. The sporidia culture readily. The echinulate chlamydospores found in two smutted heads the seeds of which were "flower-inoculated" with *U. nuda* and "seed-inoculated" with Type 3 are believed to be the F_1 between Type 3 and *U. nuda* and germinate by promycelia and sporidia which in appearance are often quite atypical.

CYTOLOGICAL STUDIES

MATERIALS AND METHODS

Ovaries of barley and wheat inoculated at flowering time with *U. nuda,* with Type 4 (Tapke collection), with *U. tritici* (Honor form), or with *U. tritici* (Reward form) were fixed at different parallel stages of development from 2 days after inoculation until maturity. As in studies on *Puccinia coronata* (Ruttle and Fraser, 1927), it was difficult to secure good fixation of the host and fungus at the same time. Four standard fixatives were used, *viz.,* chrom-acetic-urea, chrom-acetic without urea, formalin-acetic-alcohol, and chrom-acetic-formalin solution. All ovaries were opened with a sharp razor before fixation in order to allow better penetration of the solutions. Seed fixed in chrom-acetic and chrom-acetic-urea were first dipped in 95 per cent alcohol. During the 24 hours of fixation the vials were placed in a desiccator which was exhausted to 110 mm pressure. Butyl alcohol was much superior to xylol for dehydrating the material, whole almost mature seed run thru butyl sectioning readily, seed of similar age dehydrated in xylol sectioning only with extreme difficulty.

A few mature wheat seeds were soaked in chrom-acetic solution for 48 hours, dehydrated in alcohols, placed in a celloidin bath, soaked in origanum oil, then in origanum oil and paraffin, and finally embedded in paraffin by the method of Kultschitzky as described by Lee (1924, page 103). Such material sectioned especially well, altho the cells were somewhat shrunken. Some modifications of the double embedding method may have value in the study of materials such as seeds which tend to harden so as to be impossible to cut by other processes.

For a study of pericarp infection, ripe wheat and barley were soaked in water or Apathy's solution until the pericarp loosened. The pericarp was then removed, stained in Flemming's solution, and mounted in balsam or simply mounted in Linder's lacto-phenol solution to which a small portion of Ehrlich's tri-acid fuchsin was added. In the latter the mycelium when present stained bright red; no finer details, such as nuclei in the hyphae, showed.

Paraffin sections were cut 15μ in thickness and stained in Stoughton's thionin and Orange G, or in Flemming's triple stain, the latter, on the whole, giving better differentiation.

TYPE 4 (TAPKE COLLECTION)

Paraffin material of Featherston barley "blossom inoculated" with Type 4 (Tapke collection) was examined at intervals of 3, 4, 5, 7, and 10 days after inoculation and similar material of Alpha barley at intervals of 2, 4, 5, 10, and 21 days after inoculation. In the youngest material of both varieties examined spores were observed germinating by germ tubes, as occurs on cover slips in moist air, as well as by promycelia and sporidia. At 2, 3, and 4 days after inoculation hyphae were observed twining around the stigmatic branches, anthers, and pollen grains and beginning to creep along the upper surface of the ovary wall. Sporidia were frequent on the surfaces of stigmatic branches and pollen grains.

Seven days after inoculation on Featherston hyphae and sporidia were found in every cranny of the outer upper surface of the ovary wall, around the base of the ovary hairs, and between the stigma lobes.

Ten and 21 days after inoculation on Featherston and Alpha barley hyphae had extended farther down the ovary wall and were large and vigorous. No evidence of penetration could be found on either variety, altho a definite search for such penetration was made. Also, no fungus hyphae were found at any time within the ovary wall, the pericarp, the aleurone, the endosperm, or any part of the embryo. Also, pollen grains and stigmatic branches, altho twined about with thickened hyphae, were not penetrated by them.

Copulating normal-sized, as well as much-enlarged, sporidia were also observed in this material. The enlarged sporidia and thickened hyphae resemble those found in old cultures. The enlarged sporidia possibly may be monosexual, as are those which form in old monosporidial cultures, or they may be the equivalent of chlamydospores developing after the copulation of two sporidia.

Pericarps of mature Alpha seed from flowers inoculated 1½ years earlier were removed after soaking in water 1, 12, and 24 hours and were examined in lacto-phenol with Ehrlich's tri-acid fuchsin. After 1 hour of soaking, the wefts of enlarged hyphae and sporidia on the inner surface of the glumes and outer surface of the caryopsis (Fig. 6, A) still appeared dormant. After 12 hours of soaking, sprouting of both sporidia and hyphae was evident (Fig. 6, B), and after 25 hours normal-sized sporidia had formed and were multiplying by budding (Fig. 6, C). No germination of spores was

FIG. 6.—HYPHAE AND SPORIDIA ON THE OUTER SURFACE OF THE PERICARP OF MATURE SEED OF ALPHA BARLEY FROM FLOWERS INOCULATED WITH TYPE 4 (TAPKE COLLECTION).

A, after soaking the seed 1 hour in water; B, after 12 hours; and C, after 24 hours. x 1020.

observed, the source of inoculum apparently being the resting sporidia and hyphae on the glumes and caryopsis.

All spores observed on the floral parts were definitely echinulate, altho a search for smooth ones was made because of the Type 3 heads which developed in plants inoculated with this material.

U. NUDA AND U. TRITICI

Honor wheat is susceptible to the Honor form of *U. tritici*; Reward wheat to the Reward form of *U. tritici*; and Alpha barley to *U. nuda* collected on Featherston barley. Examination of ovaries of Honor, Reward, and Alpha inoculated with the loose smut to which they are susceptible yielded results which agree in general with those obtained by Hecke (1905), Brioli (1910), Brioli and

Schikorra (1913), and Lang (1917) in barley blossom inoculated with *U. nuda* and by Falck (1908) and Lang (1909) in wheat blossom inoculated with *U. tritici*.

Thus, Honor wheat fixed 14 days after inoculation with the Honor form of *U. tritici* showed abundant mycelium in the ovary wall, a little mycelium in the aleurone, and, as yet, none in the embryo and endosperm. At various points along the ovary wall, mycelium was observed growing in from the surface where there were spores directly thru the ovary wall cells at right angles to their longitudinal axis. In Reward wheat fixed 22 days after inoculation with the Reward form, the infection was more advanced, hyphae occurring in abundance not only in the pericarp, but also in the aleurone and outer layers of the endosperm as well as in the scutellum, the hypocotyl, and even the root primordia of the embryo. No hyphae were found in the plumule. In the embryo some of the hyphae were large, vigorous, and well filled with plasma, while others were quite empty and contracted in parts. The crushed nucellar layer was filled with large ramifying hyphae. This particular Reward kernel was rather unhealthy, the endosperm somewhat lacking in starch and the embryo cells in content. The mycelial advance however appeared typical.

Alpha barley inoculated with *U. nuda* (Featherston collection) and fixed 12 days after inoculation showed abundant mycelium in the pericarp, in the crushed nucellus, in the aleurone and endosperm of the seed, and in the scutellum and hypocotyl of the embryo. As in wheat, the mycelium in the nucellus is particularly vigorous and abundant. Penetration appears to be at various points along the ovary wall, as observed by Lang (1917), and particularly at the brush end where the spores are most numerous.

The penetration of hyphae of *U. tritici* at various points along the ovary wall of wheat agrees with Lang's (1917) findings in barley, but not with those in wheat (1909). The occurrence of mycelium in the root primordia of Reward wheat agrees with the findings of Brioli and Schikorra (1913) who found mycelium in all parts of the infected barley embryo even the root primordia. Lang (1917) found no trace of mycelium in the root primordia of barley and considered that there might be a difference in infection in different seeds to be explained by the rapidity of ripening, rapid ripening allowing less complete embryo infestation.

Honor wheat fixed 14 days after inoculation was less completely infested with *U. tritici* than Alpha barley fixed 12 days after inocula-

tion with *U. nuda* which agrees with Lang's findings (1917), but does not, as Lang assumed, prove that the wheat fungus is less vigorous than the barley fungus, as evidenced by Reward.

It is of importance, also, to find whether the resistance of Honor wheat to the Reward form of *U. tritici* and of Reward wheat to the Honor form is a matter of non-infection, or partial infection, or whether seed invasion is complete and the two forms of smut unable to adjust themselves to the different growing periods of the two wheats. The same considerations hold in regard to the resistance of Tennessee Winter barley to the Featherston collection of *U. nuda.*

A superficial determination of invasion was made by the examination of pericarps mounted in Linder's lacto-phenol to which was added a little of Ehrlich's tri-acid fuchsin. Hyphae were found within the pericarp of Honor wheat blossoms inoculated with the Reward form of *U. tritici,* of Reward wheat blossoms inoculated with the Honor form, and of Tennessee Winter barley blossoms inoculated with the Featherston form of *U. nuda.* At times the hyphae were few, at other times more numerous, and formed mycelial wefts on the surface and in the deeper layers of the pericarp. Whether they extend into the embryo was not determined.

In paraffin sections of Honor wheat inoculated with the Reward form and fixed 15 days after inoculation, altho abundant mycelium was present around the pollen grains, on the brush end of the ovary, and in the furrow, only a trace of degenerating mycelium was observed within the ovary wall. In similar sections of Reward wheat fixed 11 days after inoculation with the Honor form of *U. tritici,* abundant healthy mycelium was found ramifying within and between the ovary wall cells and in places even penetrating into the aleurone. As yet none was evident in the embryo or endosperm.

Pericarps of Reward wheat inoculated with *U. nuda* and Alpha and Featherston barley inoculated with the Reward form of *U. tritici* and examined in lacto-phenol showed abundant mycelium on the surface but little if any within the pericarp layers. Examination of paraffin material of Reward wheat fixed 15 days after inoculation with *U. nuda* showed no trace of infection within the ovary walls. Similarly, no mycelium was found within the ovary wall of Alpha barley fixed 20 days after inoculation or of Featherston fixed 9 days after inoculation with *U. tritici* (Reward form) altho abundant spores and mycelium were observed on the surface.

Heavy pericarp invasion by various fungi was commonly observed in Honor wheat, especially in that grown under moist conditions. Such invasion appears greatest at the brush end and is often located between the outer epidermis and parenchyma but may extend below the chlorophyll layer and form mycelial wefts in the testa and crushed nucellar layers. Seed so infected germinate well and in general seem not to be affected by the fungi in and on the pericarp.

DISCUSSION AND SUMMARY OF CYTOLOGICAL STUDIES

The formation of resting mycelium of various fungi in and on the pericarp of oats, barley, and wheat is frequent, Mead (1932) isolating from surface-sterilized wheat kernels *Alternaria, Helminthosporium sativum, Penicillium,* certain sterile fungi having dark-colored mycelium, *Fusaria, Fusaria*-like fungi, and a yeast-like fungus. Under ordinary conditions of germination many of these fungi do not infect the seedling. In certain of those which do, the mycelium on the surface of the seed may be equally, if not of greater, importance than that within the pericarp. Thus, while Gage (1927) considered the mycelium within the pericarp of oats flower inoculated with *U. avenae* and *U. levis* as the primary source of inoculum, Zade (1922, 1924), Diehl (1925), Haarring (1930), and Zade and Arland (1933) and others of this school consider that, altho there is pericarp invasion as described by Falck (1908) and Gage, the effective inoculum is the resting mycelium between the glumes and pericarp. In Type 4 there appears to be no pericarp invasion, resting mycelium and sporidia being formed entirely on the inner surface of the glumes and outer surface of the caryopsis. These germinate with the seed and doubtless produce infection. If sporidia were used for flower inoculation in place of spores, the possibility that infection is produced by still viable ungerminated spores introduced within the glumes, as was thought to be the case in *U. avenae,* would be eliminated.

At the same time this "blossom infection in the sense of Zade" (Zade and Arland, 1933) has been shown to be not the only method of infecting oats with *U. avenae.* Working with *U. levis,* Reed and Faris (1924) secured heavy infection by applying spores to oat seed and germinating under conditions of controlled temperature, moisture, and soil acidity. Following their method with *U. avenae,* Kolk (1930) obtained 100 per cent infection and observed

the mycelium penetrating the seedling tissue. Similarly, Sampson (1929) secured heavier infection with *U. avenae* by seed than by flower-inoculation, notwithstanding which, she found that the typical resting mycelium of *U. avenae* occurred abundantly on pales of oat samples yielding over 10 per cent of smut. She also recorded (1928) that, while spores of *U. avenae* collected early in the season soon lose their viability, those collected later retain it for years which doubtless explains why ungerminated spores dispersed at blossoming time and present within the glumes are usually non-viable (Zade and others), while other spores may produce heavy infection (Kolk, 1930, and Sampson, 1929). Similarly, either spores or sporidia of Type 4 applied to seed of Alpha barley are capable of producing infection.

In consequence, field infection by Type 4 on Alpha barley is probably the sum of blossom and seed inoculation, blossom inoculation being brought about by the distribution of the spores during flowering time, their germination, and the formation of resting mycelium and sporidia within the glumes and on the surface of the caryopsis, and seed inoculation by spores distributed later and which ungerminated adhere to the glumes. Both types of inoculation produce seedling infection as contrasted with the embryo infection produced by *U. nuda.*

Whether Reward invasion by the Honor form of *U. tritici,* Honor by the Reward form, and Tennessee Winter barley by *U. nuda* is simply pericarp invasion or whether it is true embryo invasion which is offset by physiological conditions, is not determined.

In summary abundant hyphae and sporidia of Type 4 were found on the inner surface of the glumes and outer surface of the caryopsis of "flower-inoculated" seed of Alpha and Featherston barley. In no case was the mycelium observed to penetrate the ovary wall nor was it found within the wall or embryo. The resting sporidia and hyphae on the surface of the seed one and one-half years old were observed to produce sporidia within 24 hours after being put to germinate.

Honor wheat, 10 days after blossom inoculation with *U. tritici* (Honor form) showed abundant mycelium in the pericarp and a little in the aleurone. In this time the mycelium had not yet reached the embryo but was abundant in the crushed nucellar and testa layers. Penetration of the mycelium thru the ovary wall was

observed. Reward wheat, fixed 22 days after blossom inoculation with the Reward form of *U. tritici,* showed profuse mycelium in the pericarp, aleurone, and endosperm, as well as in the scutellum, hypocotyl, and root primordia of the embryo. Featherston and Alpha barley 10 days after flower inoculation with *U. nuda* showed abundant rich mycelium in the pericarp, aleurone, endosperm, and embryo.

"Flower-inoculated" Reward wheat with the Honor form of *U. tritici,* Honor inoculated with the Reward form, and Tennessee Winter barley inoculated with *U. nuda,* all showed more or less pericarp invasion by the various forms to which they are resistant. In addition to invasion of the pericarp Reward wheat 15 days after inoculation with *U. tritici* (Honor form) showed invasion of the aleurone.

GENERAL CONCLUSIONS

The variability in the recognized barley smut species and the large number of intermediate types is of considerable practical importance, particularly in New York State where a large proportion of smut in the fields is caused by these intermediate types. Several of these having loose heads, which has been the field diagnosis for loose smut, are in their final analysis seedling infecting and can be controlled by the usual methods of seed treatment. These seedling-infecting loose smuts have been confused with true blossom-infecting loose smut which can be controlled only by hot water treatment or some modification of it.

To date, germination of spores has been found to be the only reliable method of distinguishing certainly between true loose smut and border-line, seedling-infecting loose smuts, especially the paler-spored heads of Type 4 and Type 5 and the darker spored heads of Type 6.

It would seem that chemical seed treatment for barley smut could be employed to a far greater extent than it is at present and that certified seed as a means of obtaining smut-free plants would need to be resorted to only when seed treatment fails to control, that is when the majority of infection is true loose smut.

Further study is needed on barley smut types, on their field occurrence, their mode of infection, their control, their biologic forms, their host reactions, and primarily on their relationship to *U. nuda, U. hordei,* and to one another.

LITERATURE CITED

Bauch, R.

1931 Geographische Verteilung und funktionelle Differenzierung der Faktoren bei der multipolaren Sexualität von *Ustilago longissima*. *Arch. f. Protistenk.*, **75**: *101–132.*

1932 *Sphacelotheca Schweinfurthiana*, ein neuer multipolarer Brandpilz. *Ber. d. Bot. Ges.*, **50**: *17–24.*

Biedenkopf, H.

1894 *Ustilago medians*, ein neuer Brand auf Gerste. *Ztschr. Pflanzenkr.*, **4**: *321–322.*

Brefeld, O.

1888 Neue Untersuchungen über die Brandpilze und die Brandkrankheiten. *Nachr. Klub Landw. Berlin, 1577–1584, 1588–1594, 1597–1601.*

1895 Die Brandpilze II. *Untersuch. aus dem Gesamtgebiete der Mycologie*, **11**: *1–92.*

1903 Neue Untersuchungen und Ergebnisse über die natürliche Infektion und Verbreitung der Brandkrankheiten des Getreides. *Nachr. Klub. Landw. Berlin*, **466**: *4224–4234.*

Brefeld, O., and Falck, R.

1905 Die Blüteninfektion bei den Brandpilzen und natürliche Verbreitung der Brandkrankheiten. *Untersuch. aus dem Gesamtgebiete der Mycologie*, **13**: *1–70.*

Brioli, J.

1910 Versuche mit Brandinfektion zur Erzielung brandfreier Gerstenstämme. *Naturw. Ztsch. Forst. u. Landw.*, **8**: *335–344.*

Brioli, J., and Schikkorra, W.

1913 Beiträge zur Biologie des Gerstenflugbrandes (*Ustilago hordei nuda* Jen.). *Ber. d. D. Bot. Ges.*, **31**: *336–339.*

Buller, A. H. R.

1930 The biological significance of conjugated nuclei in *Coprinus lagopus* and other *Hymenomycetes*. *Nature*, **126**: *686–689.*

1931 Researches on Fungi. *New York: Longmans, Green & Co.* (Vol. IV, page 329.)

Campagna, E.

1925 "New white smut" of wheat collected at Ste. Anne de la Pocatière. *Ann. Rpt. Que. Soc. Pro. Plants*, **18**: *71–72.*

Dickinson, S.

1926 A method of isolating and handling individual spores and bacteria. *Proc. Roy. Soc. Med., Sec. Path.*, **19**: *1–4.*

1927 Experiments on the physiology and genetics of the smut fungi. Hyphal fusion. *Proc. Roy. Soc. London, Ser. B*, **101**: *126–136.*

1932 The nature of saltation in *Fusarium* and *Helminthosporium*. *Minnesota Agr. Exp. Sta. Tech. Bul. No. 88.*

1933 The technique of isolation in microbiology. *Phytopath.*, **23**: *357–367.*

Diehl, O.

1925 Experimentelle Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes. *Bot. Arch.*, **11**: *146–199.*

Falck, R.

1908 Die Flugbrandarten des Getreides, ihre Verbreitung und Bekämpfung. *Jour. Landw.*, **56**: *173–182.*

Faris, J. A.

1924A Physiologic specialization of *U. hordei*. *Phytopath.*, **14**: *537–557.*

1924B Factors influencing infection of *Hordeum sativum* by *Ustilago hordei*. *Amer. Jour. Bot.*, **11**: *189–213.*

Gage, R. G.

1927 Studies of the life history of *Ustilago avenae* (Pers.) Jens. and of *Ustilago levis* (Kell. and Sw.) *Magn. Cornell University Agr. Exp. Sta. Mem. No. 109.*

Coldschmidt, V.

1928 Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* Pers.) *Zeitschr. Bot.*, **21**: *1–90.*

Grevel, F. K.

1930 Untersuchungen über des Vorhandensein biologischer Rassen des Flugbrandes des Weizens. *Phytopath. Zeitschr.* **2**: *209–256.*

Haarring, F.

1930 Eine Infektionsmethode für Haferflugbrand (*Ustilago avenae* Jens.) und ihre Anwendung zu Beiz- und Immunitätsversuchen im Laboratorium und Feld. *Bot. Arch.*, **29**: *444–473.*

Hanna, W. F.

1928 A simple apparatus for isolating single spores. *Phytopath.*, **18**: *1017–1021.*

1929 Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. *Phytopath.*, **19**: *415–452.*

Hanna, W. F., and Popp, W.

1932 Physiologic forms of loose smut of wheat. Abstract in *Phytopath.*, **22**: *11.*

Hecke, L.

1904 Ein innerer Krankheitskeim des Flugbrandes im Getreidekorn. *Ztschr. f. d. Landw. Versuchsw. in Österr.*, **7**: *59–64.*

1905 Zur Theorie der Blüteninfektion des Getreides durch Flugbrand. *Ber. d. D. Bot. Ges.*, **23**: *248–250.*

Hedrick, U. P.

1932 Loose smut in barley. *New York State Agr. Exp. Sta., 51st Ann. Rpt.*: *43.*

Herzberg, P.
1895 Vergleichende Untersuchungen über landwirtschaftlich wichtige Flugbrandarten. *Zopf, W., Beitr. z. Phys. u. Morph. niederer Organismen,* **5**: *1–36.*

Holton, C. S.
1932 Studies in the genetics and cytology of *Ustilago avenae* and *Ustilago levis. Minnesota Agr. Exp. Sta. Tech. Bul. No. 87.*

Horsfall, J. G.
1932 Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. *New York State Agr. Exp. Sta. Bul. No. 615.*

Hüttig, W.
1931 Über den Einfluss der Temperatur auf die Keimung und Geschlechterverteilung bei Brandpilzen. *Ztsch. Bot.,* **24**: *529–577.*

Jensen, J. L.
1888 The propagation and prevention of smut in oats and barley. *Jour. Roy. Agr. Soc. England. II,* **24**: *397–415.*

Johnson, A. S.
1914 Experiments in control of certain barley diseases. Abstract in *Phytopath.,* **4**: *46.*

Kellerman, W. A., and Swingle, W. T.
1890 Report on the loose smut of cereals. *Kansas Agr. Exp. Sta., 2d Ann. Rpt.,* 1889: *213–288.*

Kirby, R. S.
1927 Diseases of small grains. *New York State Col. Agr. Ext. Bul. No. 157.* (Revised July 1930.)

Kniep, H.
1919 Untersuchungen über den Antherenbrand (*Ustilago violacea* Pers.) Ein Beitrag zum Sexualitätsproblem. *Ztsch. Bot.,* **11**: *257–284.*

1926 Über Artkreuzungen bei Brandpilzen. *Ztschr. Pilzk. N. F.,* **5**: *217–247.*

Kolk, L. A.
1930 Relation of host and pathogen in the oat smut, *Ustilago avenae. Bul. Torrey Bot. Club.* **57**: *443–507.*

Lang, W.
1909 Die Blüteninfektion beim Weizenflugbrand. *Centrabl. Bakt. II,* **25**: *86–101.*

1917 Zur Ansteckung der Gerste durch *Ustilago nuda. Ber. d. D. Bot. Ges.,* **35**: *4–20.*

Lee, A. B.
1924 The Microtomist's Vade-mecum. *Philadelphia: P. Blakiston and Son, Ed. 8.*

Leukel, R. W.
1932 Factors affecting the development of loose smut in barley and its control by dust fungicides. *United States Dept. Agr. Tech. Bul. No. 293.*

Mead, H. W.
1932 Studies of methods for the isolation of fungi from wheat roots and kernels. *Sci. Agr.,* **13**: *304–312.*

Nahmmacher, J.
1932 Beitrag zur Immunitätszüchtung der Gerste gegen *Ustilago nuda* forma spec. *hordei. Phytopath. Zeitschr.* **4**: *597–630.*

Newton, M., and Johnson, T.
1927 Color mutations in *Puccinia graminis tritici* (Pers.) Erikss and Henn. *Phytopath.,* **17**: *711–725.*

Reed, G. M. and Faris, J. A.
1924 Influence of environal factors on the infection of sorghum and oats by smuts. *Amer. Jour. Bot., I,* **11**: *517–539. II,* **11**: *579–599.*

Rodenhiser, H. A.
1926 Physiologic forms of *Ustilago nuda* and *Ustilago tritici. Phytopath.,* **16**: *1001–1007.*
1928 Physiologic specialization in some cereal smuts. *Phytopath.,* **18**: *955–1003.*

Ruttle, M. L. (Ruttle-Nebel by error.)
1933 Comparative studies of field collections of *Ustilago hordei* and *U. nuda.* Abstract in *Phytopath.,* **23**: *31.*

Ruttle, M. L.
1934 Studies on barley smut in 1933. Abstract in *Phytopath.,* **24**: *16.*

Ruttle, M. L., and Fraser, W. P.
1927 A cytological study of *Puccinia Coronata* Cda. on Banner and Cowra 35 oats. *Univ. Calif. Publ. Bot.,* **14**: *21–72.*

Sampson, K.
1928 The biology of oat smuts. I. Variability of chlamydospores. *Ann. Appl. Biol.,* **15**: *586–612.*
1929 II. Varietal resistance. *Ann. Appl. Biol.,* **16**: *65–85.*

Stakman, E. C., Christensen, J. J., Eide, C. J., and Peturson, B.
1929 Mutation and hybridization in *Ustilago zeae.* I. Mutation. II. Hybridization. *Minnesota Agr. Exp. Sta. Tech. Bul. No. 65.*

Tapke, V. F.
1932 An undescribed loose smut of barley. *Phytopath.,* **22**: *869–870.*

Tisdale, W. H.
1923 An effective method of inoculating barley with covered smut. *Phytopath.,* **13**: *551–554.*

Tisdale, W. H., and Griffiths, M. A.
1927 Variants in *Ustilago nuda* and certain host relationships. *Jour. Agr. Res.*, **34**: *993–1000.*

Tisdale, W. H., and Leukel, R. W.
1924 Further studies on new seed disinfectants. Abstract in *Phytopath.*, **14**: *43.*

Tisdale, W. H., and Tapke, V. F.
1924 Infection of barley by *Ustilago nuda* through seed inoculation. *Jour. Agr. Res.*, **29**: *263–287.*

Tisdale, W. H., Taylor, J. W., and Griffiths, M. A.
1923 Experiments with hot water, formaldehyde, copper carbonate, and chlorophol for the control of the barley smuts. *Phytopath.*, **13**: *153–160.*

Vanderwalle, R.
1932 Contribution a l'étude des maladies charbonneuses de l'Orge. *Bul. de l'Inst. agronom. et des Stat. de Rech. de Gembloux.*, **1**: *1–32.*

Zade, A.
1922 Experimentelle Untersuchungen über die Infektion des Hafers durch den Haferflugbrand (*Ustilago avenae* Jens.) *Fühlings Landwirtsch. Zeitg.*, **71**: *393–406.*

1924 Neuere Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes (*Ustilago avenae* (Pers.) Jens.) *Angew. Bot.*, **6**: *113–125.*

Zade, A., and Arland, A.
1933 The relation of host and pathogen in *Ustilago avenae*; a reply. *Bul. Torr. Bot. Club*, **60**: *77–87* (trans.).